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The Origin of Cortical Vesicles and their Role in Egg Envelope Formation in the "Spiny" Eggs of a Calanoid Copepod, *Centropages velificatus*

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Abstract. The mature oocytes of the marine calanoid copepod, *Centropages velificatus*, contain two morphologically distinct populations of cortical vesicles that undergo sequential exocytoses at the time of spawning. The contents of the primary cortical vesicles are released first and form the primary egg envelope. This is followed by the exocytosis of the secondary cortical vesicles. These contain numerous intracisternal granules that, upon release into the perivitelline space, transform into a mass of fine fibers. The continual accumulation of fibers constitutes an extracellular matrix between the primary envelope and the egg's plasmalemma. Further amassment of the fibers beneath the primary egg envelope results in the formation of long, spiny projections. The evolution of the cortical vesicles was traced to the early vitellogenic oocytes and appears to be unique. The two populations of cortical vesicles are synthesized together within the same cisternal elements of rough endoplasmic reticulum (RER). The RER originates from membranous blebs off both the nuclear membrane and stacks of annulate lamellae in the early vitellogenic oocytes. Numerous intracisternal granules are present within the RER. Some of these granules fuse, forming a dense, ring-like structure in the extremities of the cisternae. These bud off from the RER to become the primary cortical vesicles. The unfused intracisternal granules remain as discrete bodies within irregular profiles of vesicular ER and comprise the secondary cortical vesicles.

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Introduction

The subject of post-embryonic development in free-living copepods has been a favorite research topic for decades. Consequently, the literature abounds with descriptions of naupliar and copepodid developmental stages. However, studies relating to embyronic development, *i.e.*, those stages from spawning to the emergence of the first nauplius, are limited to only a few early publications (Grobben, 1881; Fuchs, 1914; Witschi, 1934; Marshall and Orr, 1954, 1955). In particular, details of the mechanisms of fertilization and egg envelope formation have yet to be elucidated in the Copepoda.

Some marine calanoid species spawn their eggs into ovisacs that remain attached to the female until the emergence of the first or second naupliar stage. The majority of marine calanoids, however, are broadcast spawners, releasing the eggs freely into the surrounding water where they undergo development. Within this latter group, eggs of a variety of shapes and sizes and with different types of surface ornamentation have been observed (Johnson, 1967; Koga, 1968; Kasahara *et al.*, 1974; Uye, 1983; Marcus, 1990). The egg surfaces of most species are smooth, but others may be adorned with flanges or spines of varying shapes and lengths. The production of spiny eggs has been reported for numerous species, *Acartia tonsa* (Zillioux, 1969), *Centropages ponticus* (Sazhina, 1968), *C. hamatus* (Pertzova, 1974), *Pontella mediterranea* (Sazhina, 1968; Grice and Gibson, 1981; Santella and Ionora, 1990), *A. erythraea, C. yamadai, C. abdominalis* (Kasa-

hara *et al.*, 1974), *A. steuri* (Uye, 1983), *C. velificatus* (Marcus, pers. obs.), *Calanus glacialis* (J. Runge and Blades-Eckelbarger, pers. obs.), and *Candacia pachydactyla* (Blades-Eckelbarger, pers. obs.).

Some of the species listed above produce two morphological types of eggs where the spiny form represents a diapause stage (hatching is delayed), and the smooth form typifies a subitaneous stage (no mandatory delay in hatching) *e.g., Centropages hamatus* (Pertzova, 1974) and *C. ponticus* (Sazhina, 1968). *Pontella mediterranea* produces three morphotypes; diapause eggs with long spines, and subitaneous eggs that are either smooth or adorned with short spines (Sazhina, 1968; Grice and Gibson, 1981; Santella and Ionora, 1990). *Acartia tonsa* (Zillioux, 1969) and *A. steuri* (Uye, 1983) have been reported to produce both smooth and spiny eggs, but their physiological classification as diapause or subitaneous is still in question. For the remaining species, only spiny eggs have been observed, and there is no evidence to suggest that they are a diapause stage.

While conducting a morphological survey of copepod eggs found in sea bottom muds, we became intrigued with the spiny modifications of the egg envelopes of some species. Consequently, we initiated a study using light microscopy along with scanning and transmission electron microscopy to investigate the stages of egg envelope development and spine formation in the eggs of *Centropages velificatus*.

Materials and Methods

Adult female *Centropages velificatus* (De Oliveira, 1947) were sorted from plankton tows collected approximately 10 miles due east off the coast of Fort Pierce, Florida. Female's carrying mature, pigmented oocytes were placed in small dishes with filtered seawater that were observed every few minutes for spawned eggs. The eggs were carefully picked up by drawn-out pipettes and placed onto pieces of 35 μm mesh supported by Beem capsules (Flood, 1973). The Beem capsules sat in shallow glass dishes containing 2.5% glutaraldehyde in filtered seawater.

For transmission and scanning electron microscopy (TEM and SEM), eggs in varying stages of development, from polar body extrusion to advanced spine formation, were collected and fixed in this manner. After approximately 100 eggs were placed in a Beem capsule, the capsule was transferred to a 5% Karnovsky's (1965) glutaraldehyde-paraformaldehyde mixture in 0.1 *M* Sorensens phosphate buffer. The capsules were flushed several times with the latter fixative to prevent precipitate caused by seawater mixing with the phosphate buffer. As a matter of convenience, due to the long duration of the complete fixation process, the eggs were held in the Karnovsky's fixative for varying times depending on the time of day collected. Those collected in the morning were held at room temperature for 3 to 6 h. Those collected in the evening were held overnight at 4°C. The lower temperature slows the fixation process. There were no apparent differences in cell or organelle structure among the varying times and temperatures.

Adult females carrying mature oocytes were prepared also for TEM. Initially each individual was placed in a small amount of the Karnovsky's glutaraldehyde mixture for approximately 15 min. The head and urosome were then removed with a sharp razor and the metasomes transferred to a vial containing fresh fixative and held for the same range of times as the eggs.

This primary fixation of both eggs and adult females was followed by 2 or 3 rinses in 0.1 *M* Sorensen's phosphate buffer (pH 7.4) and then held in 2% OsO_4 in 0.1 *M* Sorensen's buffer at room temperature for 1–2.5 h. The samples were rinsed briefly with buffer and dehydrated through an ascending series of alcohols to 70%. At this point, some of the eggs were pipetted onto SEM stubs covered with double-sided sticky tape and allowed to air dry in a desiccator for 2 to 3 days. The air-dried stubs were coated with gold palladium and observed with a Zeiss Novascan 30 SEM.

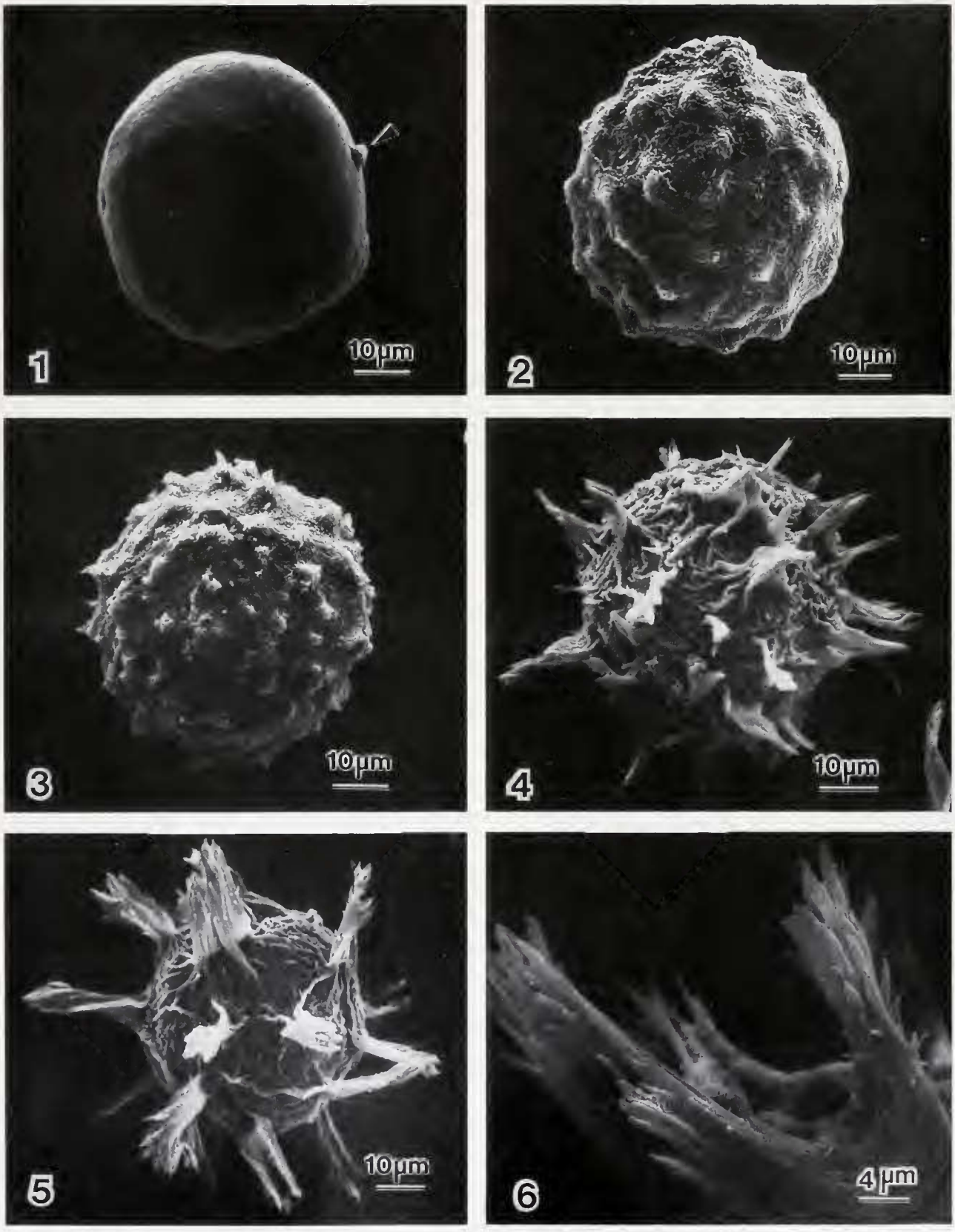
For TEM examinations, the remaining eggs and female metasomes were dehydrated further to 100% ETOH followed by propylene oxide and infiltrated with three changes of Epon (Luft, 1961). For final embedding, the female metasomes were oriented in flat embedding molds. The eggs were carefully drawn into a wide bore pipette with fresh Epon and dropped into a Beem capsule, which was centrifuged at room temperature for 20 min at setting #6 in a clinical centrifuge. Because the Epon is of a slightly thickened consistency, centrifugation is needed to concentrate the hardened eggs into the tip of the Beem capsule. Extensive sectioning of eggs prepared in this manner revealed no membrane or organelle damage. For light microscopy, 1-μm thick sections were cut with glass knives on a Porter-Blum MT2B ultramicrotome, and stained with Richardson's stain (Richardson *et al.*, 1960). Thin sections for TEM were stained with uranyl acetate followed by lead citrate and examined on a Zeiss EM9-S2 TEM.

It should be noted that the procedures for both SEM and TEM result in minor shrinkage of the eggs. Therefore, all measurements are approximations.

Results

Live observations of spawning and spine formation

Females were observed spawning on several occasions, during which they remained active, swimming in a normal manner around the dish. The oocytes flowed out of one



Figures 1–5. SEMs of egg envelope and spine formation from emergence of first polar body (Fig. 1, unlabeled arrow) to 24-h-old embryo (Fig. 5).
Figure 6. SEM. High magnification of spines.

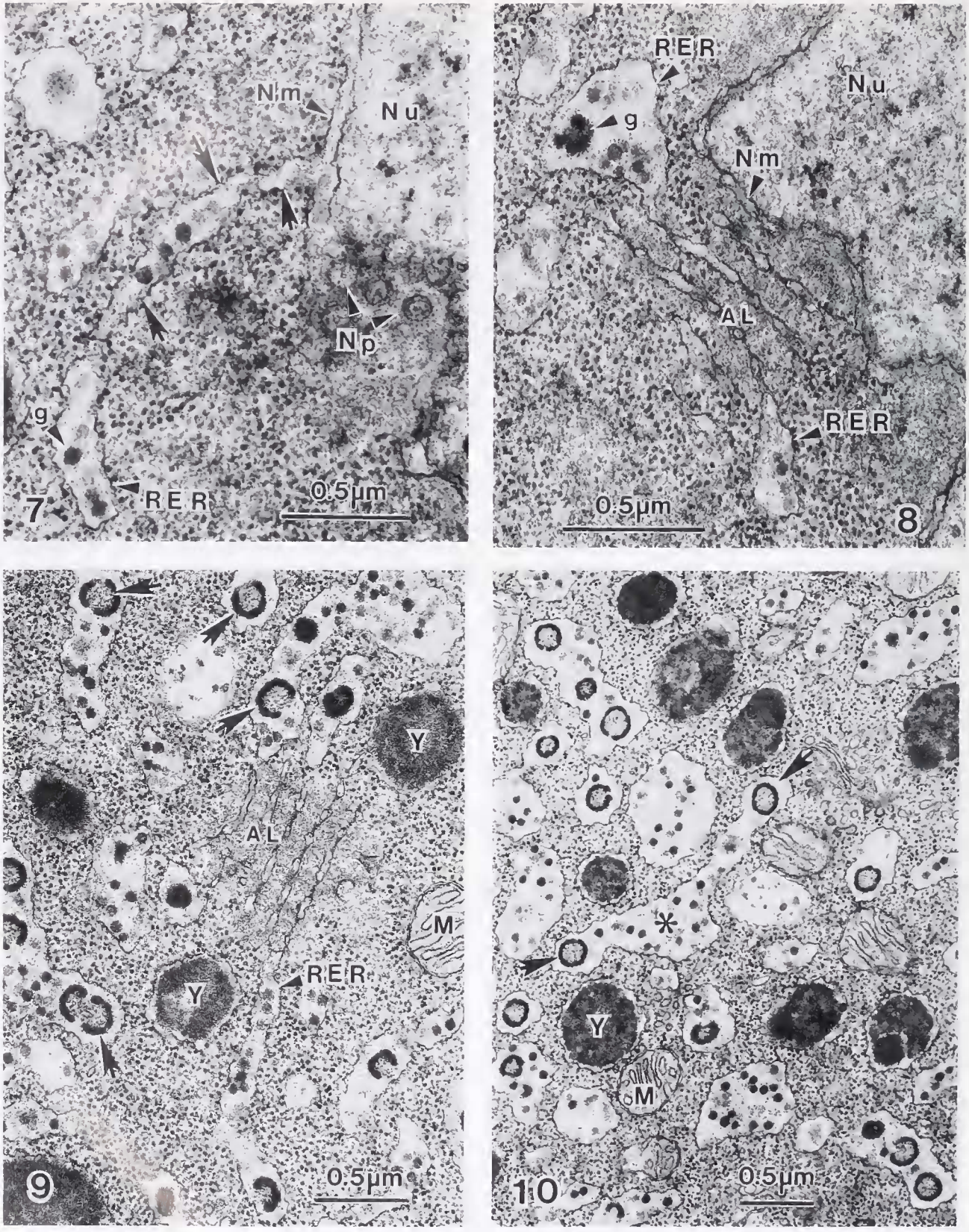


Figure 7. Perinuclear region of vitellogenic oocyte showing nuclear bleb (large arrowheads) extending from nuclear envelope (Nm) in formation of rough endoplasmic reticulum (RER). Note intracisternal granules (g) within nuclear bleb and RER. NP, nuclear pores.

Figure 8. Perinuclear region of vitellogenic oocyte showing annulate lamellae (AL). Note swollen extremities (RER) containing granules (g). Nm, nuclear membrane; Nu, nucleus.

or both oviducts, emerging from the genital pore as a single mass. The female would periodically twitch the urosome, causing the amorphous mass of eggs to break free and fall to the bottom of the dish. Approximately 5–10 s after release from the female, the eggs separated from each other and transformed from an oval to a spherical shape. Release of the first polar body occurred at this time (Figs. 1, 14). The second polar body was not observed. The actual process of sperm and egg fusion in copepods has never been reported, nor was it seen in the present study. Therefore, it could not be ascertained when egg envelope formation began relative to the moment of fertilization.

Figures 1 to 6 present comparative SEM views of the stages of spine formation from emergence of the first polar body (Fig. 1) to a 24-h-old embryo (Fig. 5). Approximately 5 min after spawning, large, rounded bumps appeared on the egg surface (Fig. 2). These bulges became more slender and pointed, forming short jagged spines (Fig. 3). It took approximately 15–20 min for long spines to form. A survey of over 100 eggs that were at least 24 h old revealed individual variations in the morphology, number, and size of the spines.

Cortical vesicle formation in vitellogenic oocytes

Formation of the egg envelope in *Centropages velificatus* involves the exocytosis of two morphologically distinct, membrane-bound inclusions present in the egg's cytoplasm. Prior to spawning, the mature oocytes that reside in the oviducts of the female contain a variety of morphologically distinct granules, vesicles, and inclusions. One type of inclusion, referred to here as the *primary cortical vesicle,* appears as a membrane-bound body containing an electron-dense, granular material that surrounds an electron-lucent core (Figs. 11, 12). Favorable sections through the center of these vesicles present the appearance of a darkly staining ring around a flocculent center (Fig. 12). The *secondary cortical vesicles* appear as irregularly shaped vesicles filled with several moderately dense granules (*ca.* 75–82 nm diameter) (Figs. 11, 12).

Primary and secondary cortical vesicles originate in the very early stages of vitellogenesis, where a blebbing process of the outer lamina of the nuclear membrane is observed (Fig. 7). These nuclear blebs contain numerous moderately dense granules (*ca.* 80 nm diameter) and pinch off to form lamellar and vesicular profiles of rough endoplasmic reticulum (RER). Stacks of annulate lamellae are also observed in the perinuclear region (Fig. 8), as well as in the central cytoplasmic region of mid- and late-vitellogenic stages (Fig. 9). Vesicles containing several dense granules, morphologically identical to the nuclear blebs, also pinch off from the extremities of the annulate lamellae. Fusion of some, but not all, of these intracisternal granules culminates in the formation of the ring-shaped densities that characterize the primary cortical vesicles (Figs. 8–10).

The cytoplasm of mid-vitellogenic oocytes is filled with elongate profiles of RER containing numerous unfused, intracisternal granules residing with one or more ring-shaped densities (Figs. 9, 10). Small Golgi complexes were observed infrequently, but did not appear to contribute to the contents of the RER. In the mature oocytes, the ring-shaped portions bud off from the RER to become the primary cortical vesicles (Figs. 10, 11). They are enclosed by a smooth membrane devoid of ribosomes. The unfused intracisternal granules remain as discrete bodies within irregular profiles of vesicular ER that also have lost the attached ribosomes. These represent the secondary cortical vesicles (Figs. 11, 12).

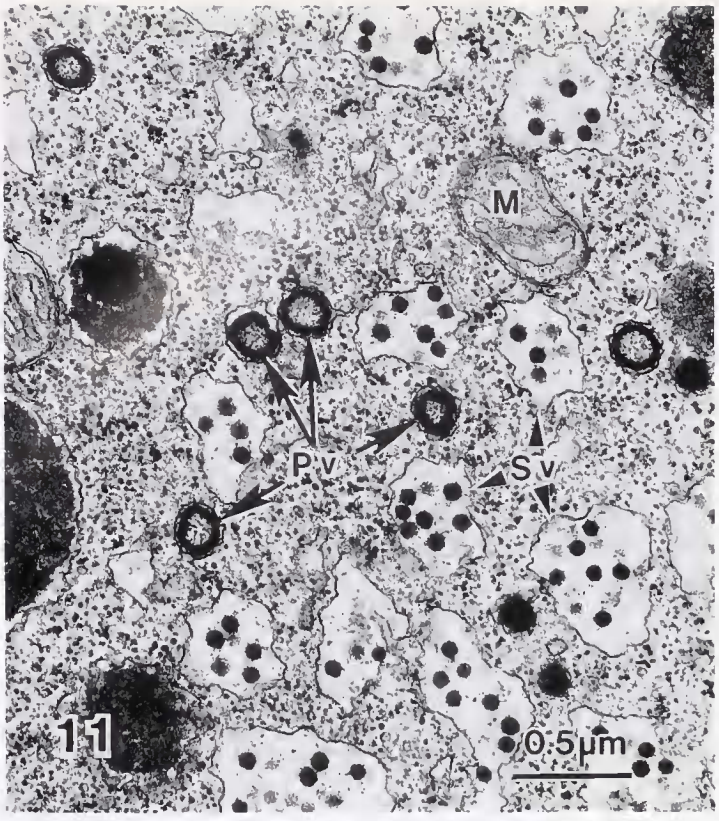
There is no elaboration of an egg envelope prior to spawning. The oocytes are enclosed by a simple oolemma that is coated with a lightly staining glycocalyx (Fig. 13). The glycocalyx, or vitelline envelope, is deposited over the oolemma by the associated follicle cells during the mid-stages of vitellogenesis (Blades-Eckelbarger and Youngbluth, 1984).

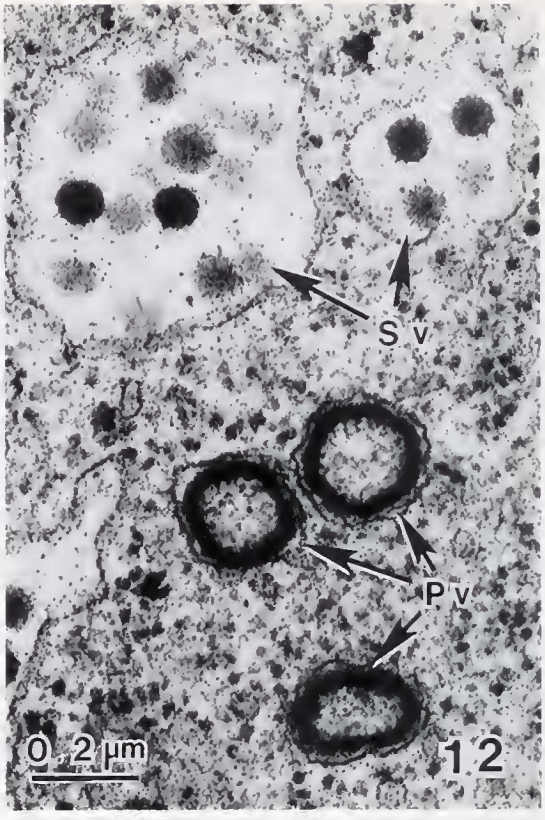
Cortical reaction, egg envelope elaboration, and spine formation

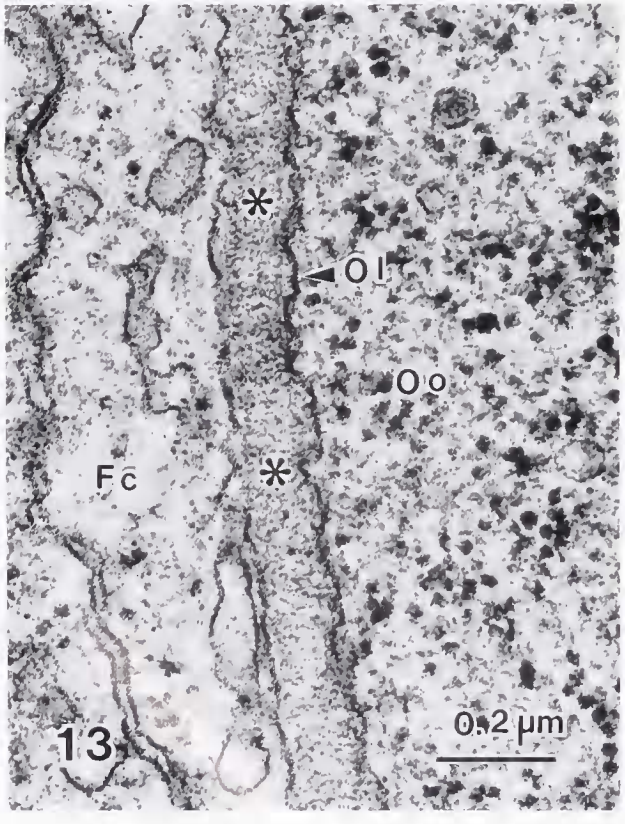
Deposition of the egg envelope results from a cortical vesicle reaction involving two successive stages of exocytosis. Soon after spawning, the majority of yolk bodies and other inclusions accumulate toward the center of the egg, but the primary and secondary cortical vesicles remain in the cortical cytoplasm (Fig. 14). The first cortical reaction is characterized by the exocytosis of the primary cortical vesicles. The bounding membrane of the primary cortical vesicles fuses with the egg's plasmalemma, and the enclosed material is released into the perivitelline space (Figs. 15, 16). This results in the formation of a narrow layer (*ca.* 20 nm thick) of darkly staining material situated slightly above the egg's plasmalemma (Figs. 15, 16, 18, 21). We refer to this first layer as the "primary egg envelope." At this time, the egg surface has a "bumpy" appearance (Figs. 2, 3, 17) where regions of the plasmalemma

Figure 9. Early stage of primary cortical vesicle formation (large arrowheads) in vesicular RER of mid-vitellogenic oocyte. Note annulate lamellae (AL) with swollen extremities (RER). M, mitochondrion; Y, yolk granules.

Figure 10. Mid-vitellogenic oocyte. Ring-shaped densities (large arrowheads) budding off of RER (*) in formation of primary cortical vesicles. M, mitochondrion; Y, yolk granule.







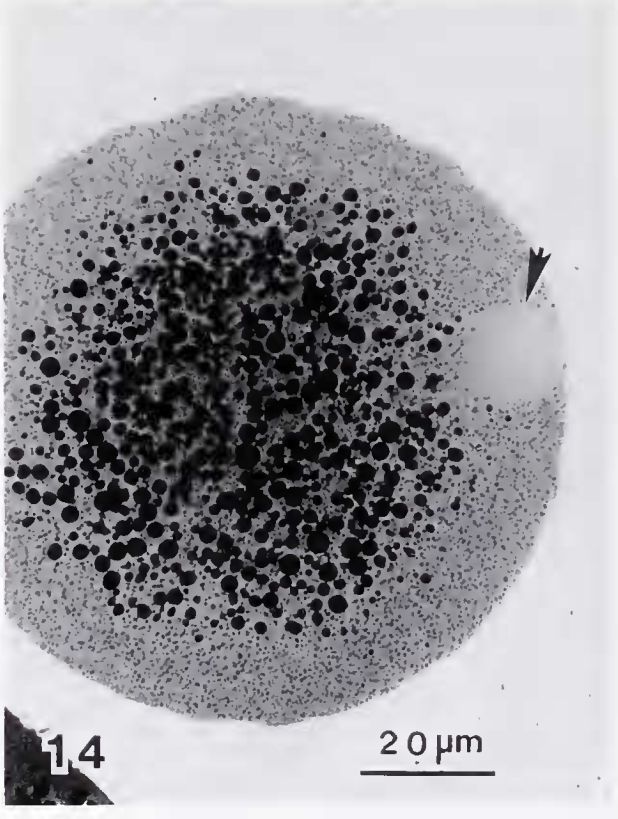
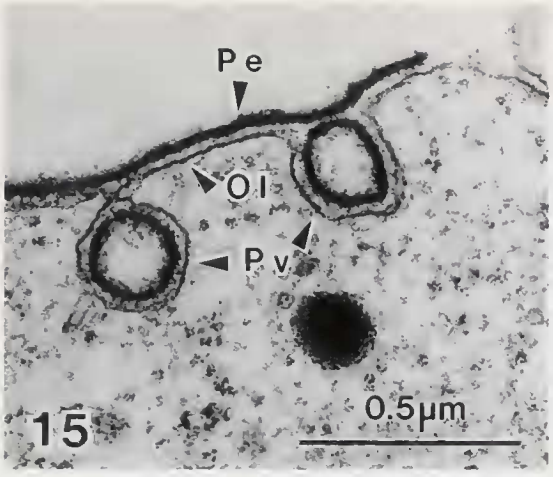
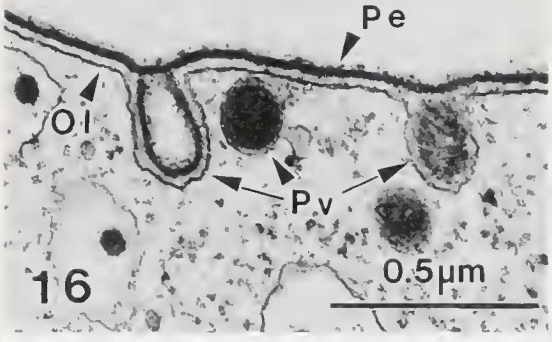
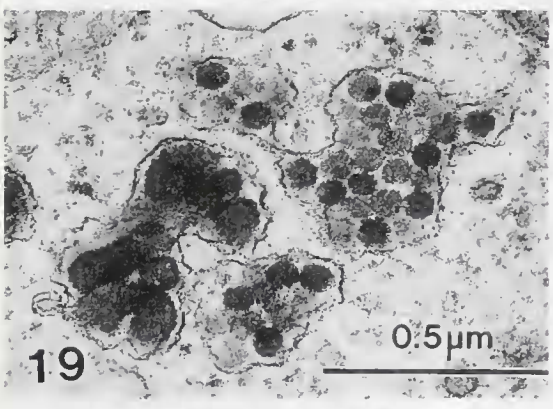
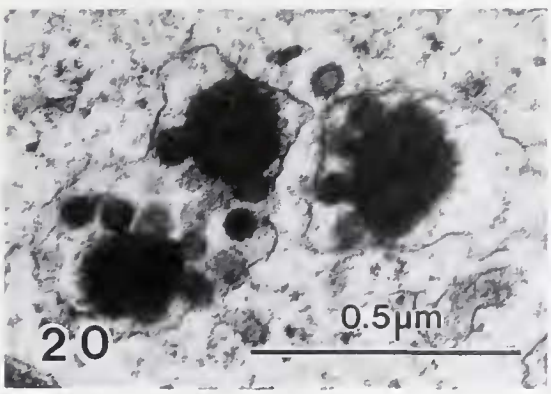


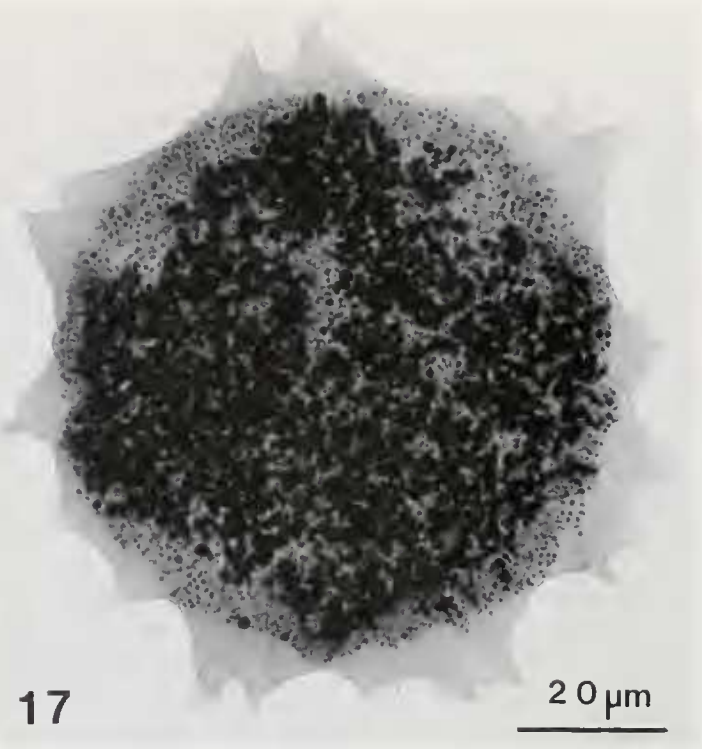
Figure 11. Late vitellogenic oocyte with primary cortical vesicles (Pv) now separate from secondary cortical vesicles (SV). M, mitochondrion.
Figure 12. High magnification showing structure of primary (Pv) and secondary (Sv) cortical vesicles.
Figure 13. Oolemma (Ol) of mature oocyte in oviduct of female covered by vitelline envelope (*). Fc, follicle cell; Oo, ooplasm.
Figure 14. Light micrograph, 1-µm-thick section of newly spawned egg and formation of first polar body (arrowhead). Note centrally located yolk granules with primary and secondary cortical vesicles occupying cortical cytoplasm.

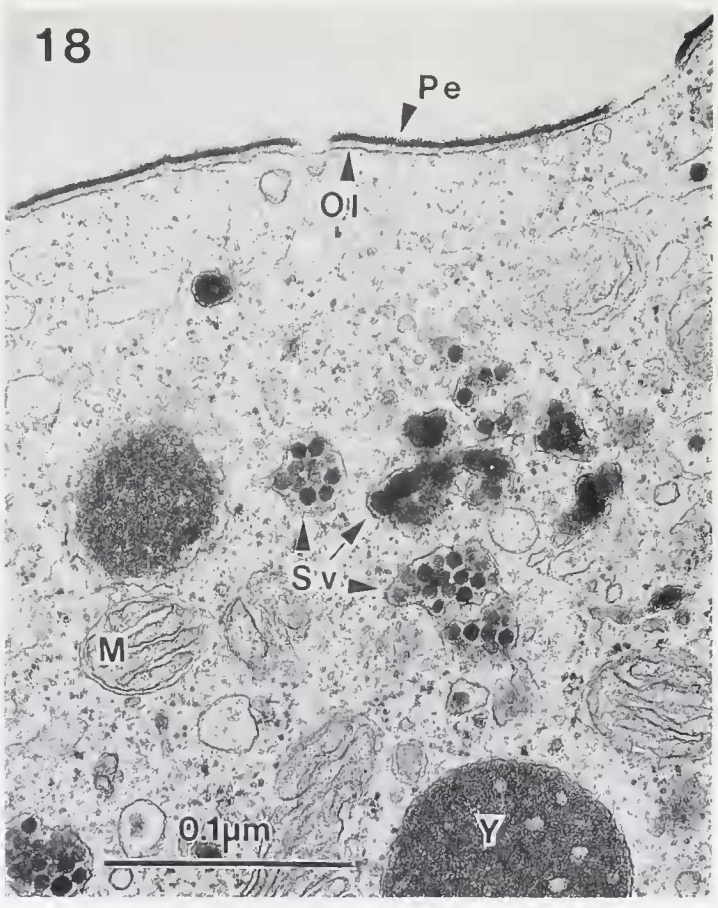












Figures 15 and 16. First cortical reaction. High magnification of egg surface showing fusion of primary cortical vesicles (Pv) with oolemma (Ol) and exocytosis of dense material in formation of primary envelope (Pe).

Figure 17. Light micrograph, 1-μm-thick section of egg during first cortical reaction and exocytosis of primary cortical vesicles. Note that cytoplasm extends into projections of egg surface.

Figure 18. Cortical cytoplasm of egg in late stage of first cortical reaction showing fusion of granules within secondary cortical vesicles (Sv). M, mitochondrion; Ol, oolemma; Pe, primary envelope; Y, yolk granule.

Figures 19 and 20. High magnification of secondary cortical vesicles showing fusion of granules.

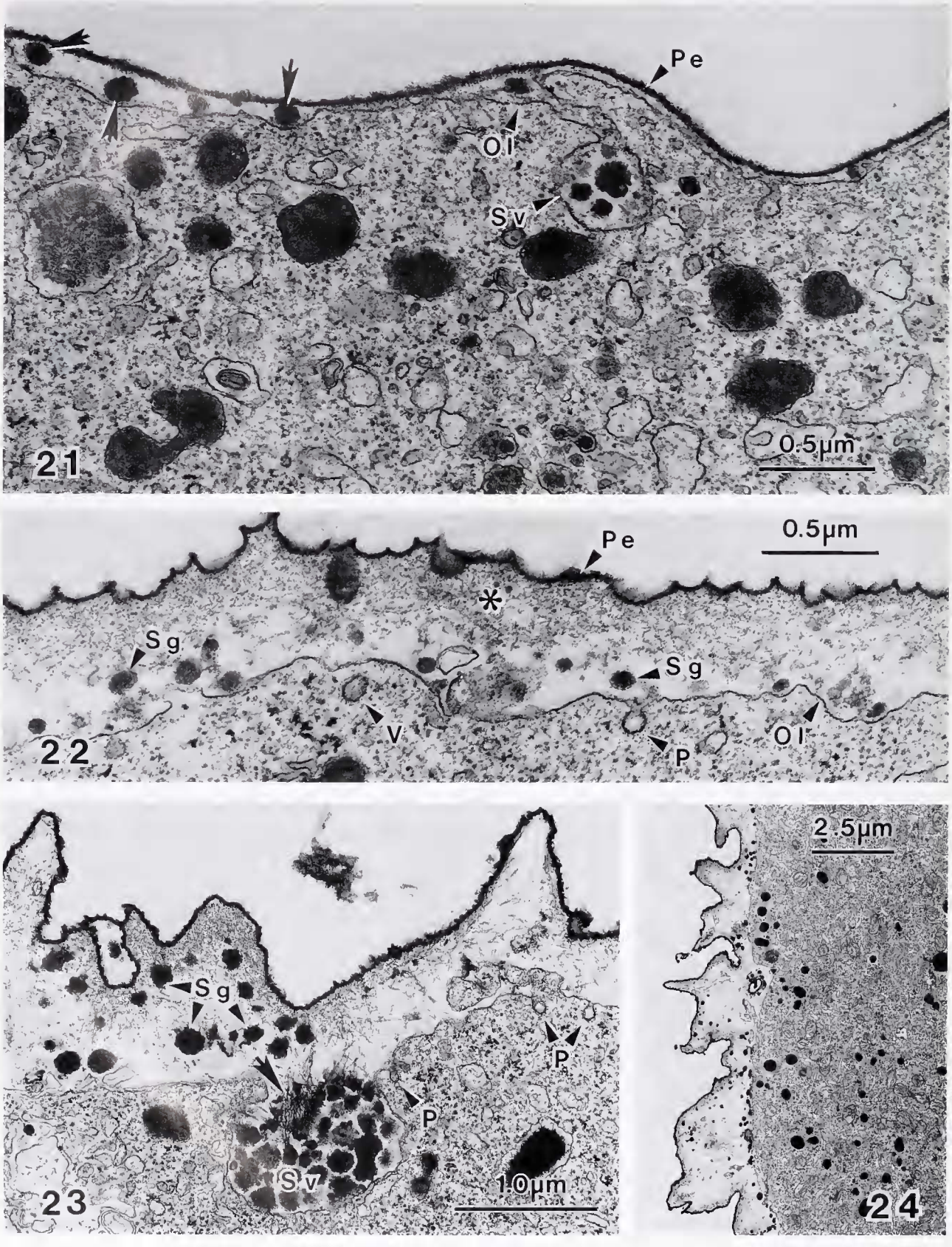


Figure 21. Second cortical reaction showing exocytosis of granules (large arrowheads) from secondary cortical vesicles (Sv). Ol, oolemma; Pe, primary envelope.

bulge out. The egg's cytoplasm projects into these expanded areas (Fig. 17).

A second wave of exocytosis follows soon after the first with the release of the intracisternal granules contained within the secondary cortical vesicles (Figs. 21, 23). Just prior to their release, however, some of the intracisternal granules fuse with each other, forming slightly larger and denser masses (Figs. 18–20). Once in the perivitelline space, the intracisternal granules transform into a meshwork of fibers that adhere to the inner surface of the primary egg envelope (Figs. 22, 23, 25, 27, 28). Concomitant with the second wave of exocytosis is the appearance of numerous endocytotic pits and vesicles along the egg's plasmalemma (Figs. 22, 23).

With the continual accumulation of fibers from the secondary cortical vesicles, the primary envelope lifts higher above the egg's plasmalemma forming an irregular surface sculpturing (Fig. 22), the plasmalemma withdraws from the core of the spines and the egg proper becomes spherical again (Fig. 26). Observations of eggs in multicellular stages (approx. 24 h), during or just after synthesis of the naupliar cuticle, revealed both long and short spines with a crenulated surface (Figs. 27, 28). The space between the cuticle and the egg envelope is composed of a thick mass of fibers (Figs. 27, 28).

Discussion

Based on our observations of the oocytes and eggs of *Centropages velificatus*, we present here the first identification of cortical vesicles, and a description of the cortical reaction and subsequent egg envelope formation in the Copepoda. These processes follow the same general sequence of post-spawning events as reported in other animal species (Schuel, 1985; Longo, 1988). However, where the eggs of some species contain a single, morphologically heterogeneous population of cortical vesicles (or granules), those of *C. velificatus* were found to have two. Consequently, the cortical reaction in the eggs of *C. velificatus*, involves not one, but two exocytotic events.

The presence of structurally different populations of cortical granules has been demonstrated in other crustaceans, the crab *Carcinus maenus* (Goudeau and Becker, 1982) and the decapod shrimp *Sicyonia ingentis* (Pillai and Clark, 1988, 1990). Talbot and Goudeau (1988) reported four distinct cortical vesicles in the oocytes of the lobster *Homarus*. In all cases, the various populations of cortical vesicles exhibited distinctly different morphologies, underwent temporally separated exocytoses, and in *S. ingentis* (Pillai and Clark, 1990) were found to be chemically heterogeneous. Each type of cortical vesicle contributed to different layers of the egg envelope.

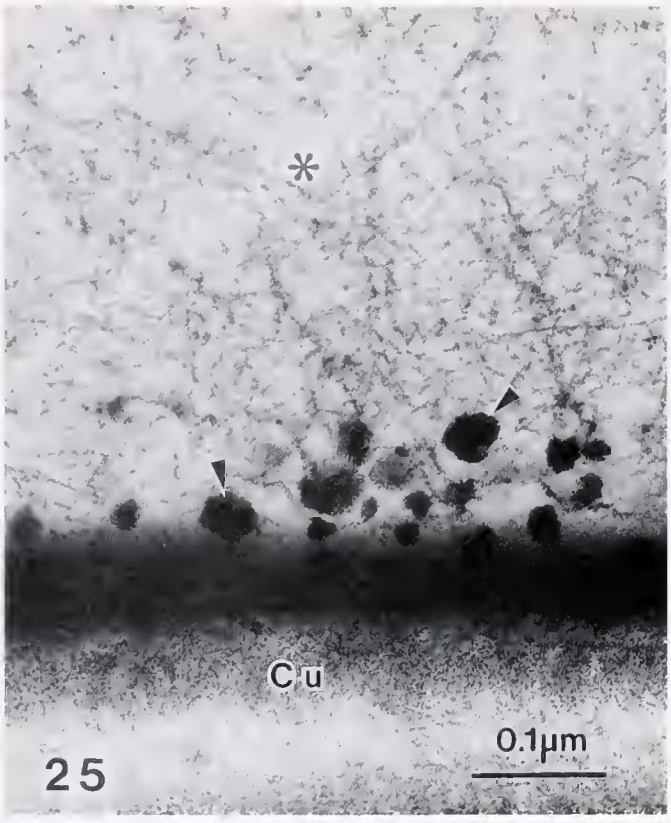
During the first cortical reaction in the eggs of *Centropages velificatus*, the contents of the primary cortical vesicles form the outer, or primary, egg envelope. This layer may correspond to the fertilization envelope of other animals, which is formed from the mixing of the vitelline layer with the exocytosed contents of the cortical vesicles (Kay and Shapiro, 1985; Somers and Shapiro, 1989). Exocytosis of the secondary cortical vesicles in the eggs of *C. velificatus* follows soon after the primary egg envelope is complete. The secondary vesicles contain several discrete intracisternal granules that, upon their release into the perivitelline space, transform into a myriad of fibers. The accumulation of these fibers between the egg's plasmalemma and the primary egg envelope forms an extracellular matrix (ECM) that exhibits a similar morphology to ECMs surrounding the eggs and embryos of other marine invertebrates (Spiegel *et al.*, 1989).

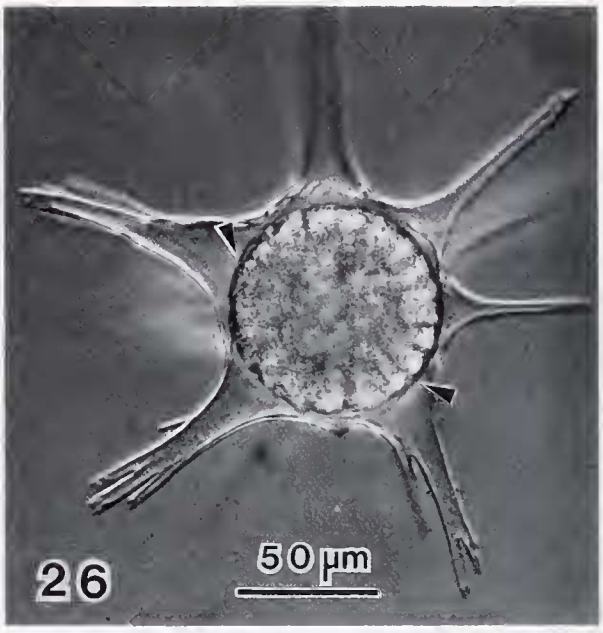
The present paper further illustrates the cellular mechanisms by which the two populations of cortical vesicles are synthesized in the vitellogenic oocytes of *Centropages velificatus*. In the oocytes of many animals, the cortical vesicles are derived from the Golgi complex (see Schuel, 1985, and references therein). In the decapod shrimp, *Sicyonia ingentis* (Pillai and Clark, 1988), one population of cortical vesicles is derived from Golgi complexes and the second population from within the cisternae of RER. Cortical vesicle formation in *C. velificatus*, in general, is similar to that of *Carcinus* (Goudeau, 1984) and *Homarus* (Kessel, 1968; Talbot and Goudeau, 1988) in which the vesicles are produced by the ER, and Golgi complexes do not appear to contribute. Other aspects of cortical vesicle formation in *C. velificatus* are unique; (1) both nuclear blebs and annulate lamellae appear to be involved in formation of the vesicular RER that synthesizes the intracisternal granules and, (2) these intracisternal granules appear to be the precursors of both the primary and secondary cortical vesicles. Fusion of some of these granules within the RER cisternae forms the dense, ring-shaped contents of the primary cortical vesicles. The other intracisternal granules do not fuse, but remain distinct and

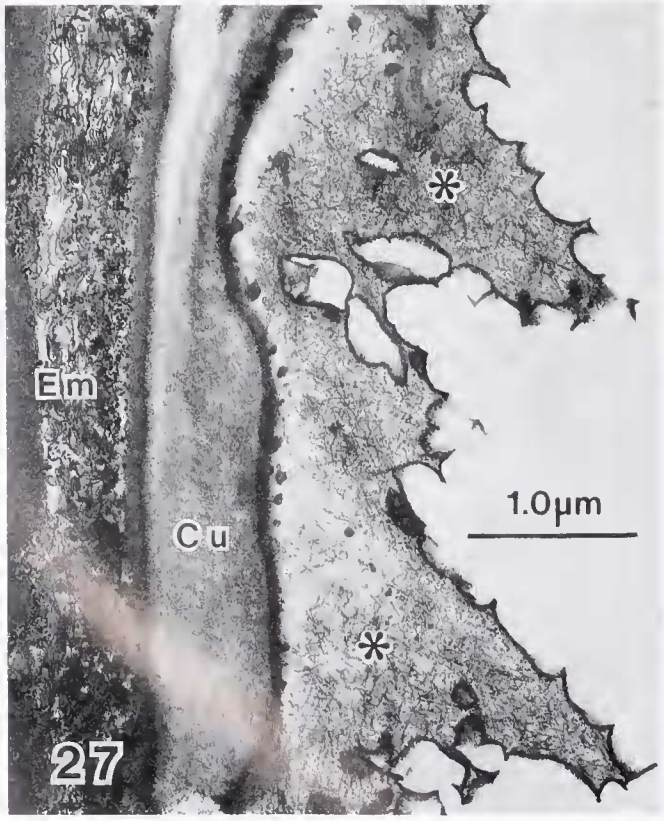
Figure 22. Second cortical reaction showing lifting of primary envelope (Pe) and filling of perivitelline space with fine fibers (*). Note surface sculpturing of primary envelope as well as coated micropinocytotic pits (p) and vesicles (v) along the oolemma (Ol). Sg, granules from secondary cortical vesicles.

Figure 23. Early spine formation showing massive exocytosis of secondary cortical vesicle (Sv). Large arrowhead denotes transformation of granular material into fine fibers. P, coated micropinocytotic pits; Sg, granules from secondary cortical vesicles in perivitelline space.

Figure 24. Mid-stage of spine formation.







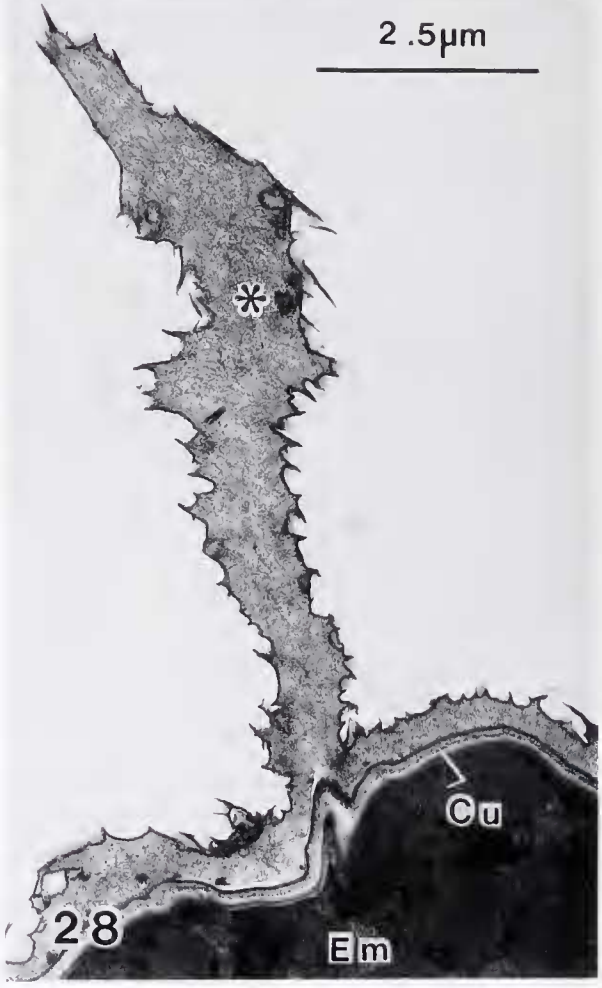


Figure 25. High magnification of perivitelline space in 24-h-old embryo showing transformation of granules (small arrowheads) from secondary cortical vesicles into fine fibers (*). Cu, early cuticle of nauplius.

Figure 26. Light micrograph of live 24-h-old embryo with advanced spine formation. Note that cytoplasm has receded from spines (small arrowheads).

Figures 27 and 28. Advanced spine formation of 24-h-old embryo (Em) showing thick mass of fibers filling spines (*). Cu, cuticle of nauplius.

comprise the secondary cortical vesicles. The primary vesicles separate from the secondary vesicles in the later stages of vitellogenesis.

In the eggs of *Carcinus maenus* (Goudeau and Lachaise, 1980a, b), the cortical vesicles of one type are filled with "ring-shaped" granules that are the precursor of the main layer of the embryonic capsule. The authors emphasized that these ring-shaped granules are homologous to the "disc-shaped granules" or "intracisternal granules" previously considered as endogenous yolk in the vitellogenic oocytes of several decapod crustaceans (Beams and Kessel, 1962, 1963; Kessel, 1968; Ganion and Kessel, 1972). Subsequent studies have confirmed that, instead of possessing nutritive qualities, the ring-shaped granules in these crustacean eggs play a structural role in formation of the egg envelope (Goudeau and Becker, 1982; Goudeau, 1984; Talbot and Goudeau, 1988; Pillai and Clark, 1990).

Within the Calanoida, the secondary cortical vesicles of *Centropages velificatus* appear homologous to the "intracisternal granules representing the endogenous yolk" in the oocytes of *Centropages typicus* (Arnaud *et al.*, 1982) and to the "granular form of type 1 yolk" in the oocytes of *Labidocera aestiva* (Blades-Eckelbarger and Youngbluth, 1984) and *Pontella mediterranea* (Santella and Ianora, 1990). Our present observations parallel those of Goudeau and Lachaise (1980a, b) and Talbot and Goudeau (1988), illustrating that the intracisternal granules previously assumed to represent endogenous yolk in copepod eggs, are actually precursors of the egg envelope. The distinctive morphology of the primary cortical vesicles in *C. velificatus,* however, has no correlate in the eggs of other copepod species studied thus far, even in the oocytes of a congeneric species, *C. typicus* (Arnaud *et al.*, 1982). The fact that the eggs produced by *C. typicus* do not elaborate spines warrants a closer look at morphological differences between the eggs of these congeners.

One consequence of the two exocytotic episodes in the eggs of *Centropages velificatus* is the addition of large quantities of membrane to the egg's plasmalemma. This occurs when the limiting membrane of the cortical vesicles fuses with the plasmalemma of the egg. However, the diameter of the egg does not increase significantly. The presence of numerous endocytotic pits and vesicles observed along the egg's plasmalemma during the second exocytotic event provides a mechanism for the recycling of at least some of the extra surface membrane. This process has been illustrated in the eggs of other animals (see review by Longo, 1988) and conforms with similar observations on mammalian secretory tissues (Mata and Christensen, 1990).

Earlier studies have described two membranes surrounding the copepod egg (see reviews by Davis, 1968, 1981). During hatching, the outer membrane cracks and the inner membrane pushes out. The outer membrane slips off and the nauplius is enclosed within the more delicate inner membrane. The nauplius then breaks open this membrane with its appendages and swims free. The ultrastructural features of the primary egg envelope in *Centropages velificatus* do not exhibit a trilamellar composition indicative of a true membrane. Therefore, we suggest that this layer should be referred to as the hatching envelope, such as described for other crustaceans (Goudeau and Becker, 1982; Pillai and Clark, 1988). The presence and structure of an inner egg membrane around the copepod nauplius has yet to be validated because we did not examine the later embryonic stages.

The morphology of the subitaneous egg envelope of *Centropages velificatus* is very different from that of the envelope encasing diapause eggs as reported for *Hemidiaptomus ingens privinciae* (Champeau, 1970), *Diaptomus sanguineus* (Hairston and Olds, 1984), *Pontella mediterranea* (Santella and Ianora, 1990), and *Anomalocera patersoni* (Ianora and Santella, 1991). The thick, multi-layered envelope surrounding diapause eggs appears as a lamellar arrangement of microfibrils in a helicoidal array and is considered comparable to the typical integument of arthropods (Hairston and Olds, 1984).

The function of the spines that characterize the eggs of *Centropages velificatus* and those of other copepod species remains elusive. During a discussion session of the symposium entitled "Cultivation of Marine Invertebrates" held in Princeton in 1967, it was suggested that spines on copepod eggs might retard sinking (Allen, 1969), enhance gas exchange, and afford protection from predation (Shelbourne, 1969). However, while it seems reasonable that the spines would deter predators, Zillioux (1969) reported that spiny eggs were consumed by adult female *Acartia.* More recently, Santella and Ianora (1990) suggested that the four-layered egg envelope and accompanying spines present on the diapause eggs of *Pontella mediterranea* may supply nutritive material during diapause and provide extra protection from harsh environmental conditions.

Numerous functions have been proposed for the spines that cover the surface of other marine invertebrate eggs. The eggs of some sea urchins, starfish, and sea anemones present a spiny appearance due to the elongation and bundling of cortical microvilli (Schroeder, 1982, 1986). It is suggested that these microvillous "spikes" and "spires" play a role in reinforcing the egg surface (Schroeder, 1982), function to facilitate absorption, and aid in adhesion between dividing cells of the embryo (Schroeder, 1986). Copepod oocytes are not known to possess microvillar modifications of the oolemma (Blades-Eckelbarger and Youngbluth, 1984), nor do the eggs form microvilli after fertilization (present study). Furthermore, the spines of *Centropages velificatus* are not cytoplasmic, but are projections of the outer or primary egg envelope caused by the amassment of an extracellular matrix (ECM) within

the perivitelline space. Recent studies on the ECMs surrounding the embryos of other marine invertebrates may hint to the role of the ECM that coats the eggs of *C. velificatus*. ECMs are believed to provide support and protection to the developing cells, aid in cell movement and cell adhesion, and form a semi-permeable filter for uptake and concentration of substances from the environment needed for growth and differentiation (Spiegel *et al.*, 1989).

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